IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Miller et al.	Examiner: Sarae Bausch
Serial No.	:	10/541,044	Art Unit: 1634
Cnfrm. No.	:	1984	
Filed	:	January 2, 2004	
For	:	HYBRIDIZATION-BASED BIOSENSOR CONTAINING HAIRPIN PROBES AND USE THEREOF	

REQUEST FOR RECONSIDERATION

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the December 30, 2009, Advisory Action, which acknowledged entry of Applicants' submissions on November 23, 2009, Applicants respectfully request reconsideration of the several remaining rejections set forth in the Office Action dated July 24, 2009.

No claims amendments have been made. Claims 1-25, 27-37, 42, 44-61, 67, and 68 remain pending, with claims 29-36 and 44-61 standing withdrawn. Claims 1-25, 27, 28, 37, 42, 67, and 68 are under examination.

This submission is accompanied by a Request for Continued Examination, along with new evidence in the form of a Second Declaration of Benjamin L. Miller under 37 C.F.R. § 1.132 ("Second Miller Declaration"), and a petition for a three-month extension of time. All fees associated with this submission should be charged to deposit account 14-1138. Any overpayment or underpayment should be applied to this same account.

As noted in the Advisory Action, the following rejections have been withdrawn: (i) the rejection of claims 37 and 42 under 35 U.S.C. § 103(a) for obviousness over U.S. Patent

No. 6,312,906 to Cass et al. ("Cass") in view of Vannuffel et al., "Specific Detection of Methicillin-Resistant *Staphylococcus* Species by Multiplex PCR," *J. Clin. Microbiology* 33(11):2864-2867 (1995) ("Vannuffel"), Berger-Bachi et al., Genbank accession No. X17688 ("Berger-Bachi") and U.S. Patent No. 5,541,308 to Hogan et al. ("Hogan"); (ii) the rejection of claim 25 under 35 U.S.C. § 103(a) for obviousness over Cass in view of Herne et al., "Characterization of DNA Probes Immobilized on Gold Surfaces," *J Am Chem Soc* 119:8916-8920 (1997) ("Herne") and further in view of Vannuffel, Berger-Bachi, and Hogan; and (iii) the rejection of claims 1-25, 27, 28, 37, 42, 67, and 68 on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 1-8, 11, 13, 14, and 17-21 of U.S. Patent No. 7,442,510 to Miller et al.

The rejection of claims 1-11, 14-21, 24, 27, 28, 67, and 68 under 35 U.S.C. § 103(a) for obviousness over Cass in view of Herne is respectfully traversed. Applicants respectfully disagree for the reasons set forth *infra* and in both the previously submitted Declaration of Benjamin L. Miller under 37 C.F.R. § 1.132 ("First Miller Declaration") and the Second Miller Declaration.

Cass generally teaches the use of surface bound *hairpin* nucleic acids for detection of target nucleic acids. While Cass describes the use of dithiols to control hairpin density, Cass provides no guidance for their use and none of the Examples illustrate their use. To be clear, it is Cass's use of dithiols that are referenced above. *See* Second Miller Declaration at ¶ 3. Thus, Cass fails to teach or suggest the binding of its disclosed dithiols and *hairpin* molecules "following exposure of the fluorescence quenching surface to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater" as recited in claim 1. Cass, therefore, fails to teach or suggest the need for using the recited ratio of spacer to *hairpin* nucleic acid molecule in preparing a sensor chip that exhibits the recited fold-increase in fluorescence.

The PTO at page 4 of the office action asserts that Herne overcomes this deficiency of Cass. Applicants respectfully disagree for several reasons.

Firstly, the schematic illustrated in Figure 4B of Herne merely shows a cartoon of the Herne substrate having a 5:1 ratio of MCH:DNA, but this does not overcome the deficiencies of Cass, because Herne never exposes the fluorescence quenching surface to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater. The

claim language does *not* recite that a ratio of spacer:nucleic acid of about 5:1 or greater exists on the substrate, but rather that the substrate is exposed to such a mixture.

Claim 1 of the present application recites a process limitation in defining the resulting product. Second Miller Declaration at ¶ 4. This process limitation concerns the "exposure of the fluorescence quenching surface to a mixture comprising a ratio of spacer molecule to first nucleic acid molecule of about 5:1 or greater." *Id.* This process limitation is used to define the resulting product, because this process limitation is what allows the hairpin probes to fold and thereby afford reduced background signaling. *Id.* The reduced background signaling is what allows the "sensor chip [to exhibit] at least a 5-fold increase in fluorescent emissions intensity when the sensor chip is exposed to a target nucleic acid molecule that hybridizes specifically to the first nucleic acid molecule" as claimed. *Id.* That this process achieves a product as claimed is demonstrated in the Examples of the present application and the comparative data presented in the First Miller Declaration at ¶ 6.

Secondly, the combination of Cass and Herne would not have arrived at the claimed invention and one of ordinary skill in the art would not have expected as much.

Herne teaches a two step method of controlling the surface coverage of thiol-derivatized *linear* DNA probes on the surface of gold substrate. In the abstract Herne states, "[t]he primary advantage of using this two step process to form HS-ssDNA/MCH mixed monolayers is that nonspecifically adsorbed DNA is largely removed from the surface." Applicants respectfully submit that Herne merely teaches a method of removing excess, nonspecifically bound DNA probes from the surface of the substrate. There is a significant difference between the dissociation kinetics involved in removing nonspecifically adsorbed DNA and the binding kinetics of attachment of DNA probes to a substrate. This difference is evident when these two events are compared in terms of probe distribution on the surface of the substrate.

The first step of Herne involves immersing the substrate in a solution of *linear* single stranded derivatized DNA probes and allowing the *linear* probes to bind to the substrate randomly, either via the thiol or by non specific adsorption. *Only* the total amount (specific + non specific) of DNA probe which gets attached to the surface is controlled by timed exposures. In other words, this binding event fails to control the ratio between the amount of thiol bound probe to the amount of non-specifically adsorbed probe. The spacer thiol, 6-mercapto-1-hexanol

(MCH) is subsequently added in the second step and functions to remove the non-specifically adsorbed DNA and to fill in the gaps left by non-specifically adsorbed DNA. In terms of reaction kinetics, the subsequent removal of non-specifically adsorbed DNA probe from the surface would not lead to redistribution of thiol bound probes on the surface. Depending upon a number of other factors, such as ionic concentration, length of DNA probes, temperature, amount of purine versus pyrimidine residues in the DNA probe the binding process could be dominated either by thiol bound DNA or by nonspecific adsorption of the DNA, and could result in, for example, variations in localized concentrations of thiol bound DNA probe. Nevertheless, the two step process described by Herne does not involve exposing the surface to the recited mixture of agents.

Also, it is well known in the art that the folding and self assembly properties of *hairpin* DNA are very different from those of *linear* DNA probes. A person skilled in the art would not be motivated to combine the teachings of Cass and Herne because of these differences. The single stranded *linear* DNA probe has exposed aromatic rings which are extremely hydrophobic and energetically favor reduced exposure of aromatic rings. Such hydrophobic interactions would force the *linear* probes to adsorb to the gold surface much more readily. On the contrary, the *hairpin* molecule is a partially double stranded molecule and would not be exposed to similar hydrophobic constraints. Also, the charge density for a collection of hairpin molecules is more than linear DNA probes, because of the double stranded nature of the hairpin, and would therefore behave differently under similar ionic conditions. Since the hairpin molecules of the present invention require unfolding in order to bind to the target molecule the spacing requirements for a *hairpin* when compared to a *linear* DNA probe would be significantly different. Consequently, one of skill in the art would have expected that the method of Herne would not work with hairpin probes.

Neither Cass, Herne, nor the combination thereof teach a process that can achieve the claimed product. Second Miller Declaration at ¶ 5. That is because the processes of Herne and Cass will *not* result in a functional product that can achieve the limitations of the claimed product. *Id*. The previously submitted experimental evidence (as discussed below) confirms that the optimal process conditions of Herne, if used with a hairpin probe, such as that taught generally by Cass, will not result in a sensor chip that exhibits "at least a 5-fold increase in

fluorescent emissions intensity when the sensor chip is exposed to a target nucleic acid molecule that hybridizes specifically to the first nucleic acid molecule" as claimed. *Id.*

The First Miller Declaration provides evidence that an expectation of success would have been lacking and that Herne does not overcome the deficiencies of Cass. Specifically, the First Miller Declaration at ¶¶ 4-7 demonstrates that a sensor chip synthesized by using the optimized synthesis steps of Herne with hairpin probes (i.e., as described by Cass) does not afford a functional sensor chip. For this reason, one of skill in the art would not have had a reasonable expectation of success in combining the teachings of Cass and Herne to arrive at the claimed invention. Moreover, the combination of Cass and Herne does not teach each and every aspect of the claimed invention because of these deficiencies.

In the Advisory Action, the PTO makes a number of assertions to support its refusal to give weight to the evidence presented in the First Miller Declaration. Applicants submit that these assertions are without basis and that the evidence presented in the First Miller Declaration demonstrates that the combination of Cass/Herne would not have achieved a product as presently claimed.

On page 4 of the Advisory Action, the PTO asserts that "Herne teaches the substrate has spacer molecules with a ratio of 5:1 and thus it would have been obvious to modify the sensor chip of Cass with the spacer molecules of Herne." This assertion of the PTO ignores the fact that one of skill in the art would be concerned with the process that Herne teaches for arriving at their schematically illustrated structure rather than the illustration *per se*. Second Miller Declaration at ¶ 6. Thus, one of skill in the art would have utilized Herne's proclaimed optimal conditions to actually undertake the introduction of Herne's spacer molecules (MCH) in conjunction with hairpin probes of the type taught in Cass. *Id.* A person of skill in the art would understand that the schematic illustration of Herne depicting a 5:1 ratio of spacer molecules to probe on the surface of the chip is nothing more than an illustration—it is not an indication that such a condition existed on the surface of the chip. *Id.* In fact, one cannot *a priori* calculate how much hairpin probe is present on the chip in the Cass/Herne case. *Id.*

On page 5 of the Advisory Action, the PTO asserts that "[t]he substrate of applicants has almost a 10:1 ratio of probe:target …while the substrate of Cass and Herne provided for by applicant is only a 2.5 fold difference…." Neither of these statements is true. Second Miller Declaration at ¶ 7. The PTO has apparently confused the concentration used to

prepare the chip (i.e., the process conditions) with the concentration used in the assay. *Id.* There is no indication how much probe remains on the chip surface following the combined Cass/Herne process. *Id.* However, whether using the process of the presently claimed invention or the sequential process of Herne, the amount of probe remaining on the surface should be substantially lower than the amount of target used during the assay (i.e., there should be a large excess of target relative to probe). *Id.* Thus, the different ratios cited by the PTO are insignificant. *Id.*

At the bottom of page 5 and top of page 6 of the Advisory Action, the PTO asserts that "...the hairpin probe provided for in the declaration is not the hairpin probe disclosed by Cass. Cass exemplifies a 5' end with fluorescein and a 3' end biotin group attached to a linker." While the PTO is entirely correct in this regard, the reason for the use of a different hairpin probe is because the Cass probe (cited by the PTO) would not under any circumstances yield a functional chip that can achieve at least a 5-fold increase in fluorescent emissions intensity when the sensor chip is exposed to a target nucleic acid molecule that hybridizes specifically to the first nucleic acid molecule. Second Miller Declaration at ¶ 8. The reason why this is true is because there is no way to attach a biotinylated DNA probe to a gold chip, unless one had some way of first attaching streptavidin to the chip. *Id.* If streptavidin were used as a first attachment layer, its size would prevent the fluorophore from being brought in proximity to the metal film, and therefore one would have the weak streptavidin-based quenching described by Cass rather than the strong metal-based quenching described in the present application. *Id.* Thus, the use of a different hairpin probe in combination with the Herne process steps afforded the best possible chance for the combination of Cass/Herne to yield a functional chip. *Id.* Even so, as explained in the First Miller Declaration (and described above), this was not enough to overcome the deficiencies associated with the chip preparation steps as taught by Cass/Herne. *See id.* The combination of Cass/Herne would not afford a sensor chip as presently claimed. *Id.*

On page 6 of the Advisory Action, the PTO concludes that the teachings of Herne are consistent with the teachings of the present application. Dr. Miller disagrees with this statement, because while both Herne and the present invention discuss the use of thiols (Herne specifies MCH and the preferred thiol in the present application is mercaptopropanol), Herne's approach for the use of the MCH is *very* different for the use of the spacer molecule in the manner as claimed. Second Miller Declaration at ¶ 9. As discussed above, Herne's sequential

use of probe and then MCH is not at all like the simultaneous exposure recited in the claims. *Id.* The significant difference is that the sequential approach taught by Herne will not afford a functional chip when using hairpin probes. *Id.* The experimental results, using the *optimal* conditions of Herne, confirm as much. *Id.* The comparative data (from the First Miller Declaration) was offered to confirm that the probe and the process limitations of claim 1 afford a functional chip. *Id.* Even discounting the comparative evidence due to the differences between the two sets of data, the evidence of record proves that the optimal conditions of Herne when combined with the use of a hairpin probe (that is otherwise capable of functioning properly), will not produce a functional chip. *Id.*

In summary, neither Cass, Herne, nor the combination thereof teach the process limitation recited in claim 1 and, consequently, the combination of Cass/Herne would not have achieved a sensor chip as recited in claim 1. Based on the experimental evidence of record, persons of skill in the art would have lacked any expectation that the combination of Cass/Herne would have arrived at the claimed product.

For all these reasons, the rejection of claims 1-11, 14-21, 24, 27, 28, 67, and 68 for obviousness over the combination of Cass and Herne is improper and should be withdrawn.

The rejection of claims 12, 13, 22, and 23 under 35 U.S.C. § 103(a) for obviousness over the combination of Cass and Herne, and further in view of U.S. Published Patent Application No. 2002/0034747 to Bruchez et al. ("Bruchez") is respectfully traversed.

The teachings and deficiencies of the combination of Cass and Herne with respect to claim 1 are noted above.

Bruchez is cited at page 6 of the office action for teaching use of semiconductor nanocrystal labels attached to different polynucleotides for simultaneous analysis. The PTO has failed to demonstrate how Bruchez overcomes the above-noted deficiencies of Cass and Herne. For this reason, the rejection of claims 12, 13, 22, and 23 for obviousness over the combination of Cass, Herne, and Bruchez is improper and should be withdrawn.

The provisional rejection of claims 1-25, 27, 28, 37, 42, 67, and 68 on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-14 of copending U.S. Patent Application Serial No. 11/838,616 to Miller et al. ("Miller I") is respectfully traversed. Although applicants submit that the claims of the later-filed application

are patentably distinct, withdrawal of this provisional rejection is nevertheless proper pursuant to *Manual of Patent Examining Procedure* § 804 (p. 800-17). Because all other remaining rejections should be withdrawn for the reasons discussed above, the provisional obviousness-type double patenting rejection of claims 1-25, 27, 28, 37, 42, 67, and 68 over claims of Miller I should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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